Epizootic study in a turbot farm: bacteriology, virology, parasitology and histology

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ABSTRACT

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During the past 5 years there has been considerable development of turbot (Scophthalmus maximus L.) farming in Galicia (Notthwestern Spain). This development caused a concomitant increase of pathological problems in this species. In this study, the preliminary results of a microbiological survey (comprising bacteria, virus and parasites) in an ongrowing turbot farm are given. The bacteria more frequently isolated belong 10 the genus Vibrio (V. splendidus-V. pelagius), and wirh less frequency to Pseudumonas. Streptococcus and Staphylococcus. Birnaviruses (IPN-like virus) were isolated from only two samples. The flagellate Costia sp., the ciliates Trichodina up. and Cryptocaryon sp.. the microsporidian Tetramicra brevifilum and the cestode Bothriocephalus scorpii all occurred in low prevalence.

INTRODUCTION

During the past 5 years there has been a considerable increase of turbot (Scophthalmus maximus L.) farming in Galicia (Northwestern Spain) reaching a production of about 350 metric tons (t) in 1989 with a production objective of 1000 t for 1991. An increase

in disease has accompanied the development of turbot culture. Until now, vibriosis has been one of the most threatening bacterial diseases of fish culture in sea water in Galicia. Whereas Vibrio anguillarum serotypes 01 and 02 are mainly responsible for mortality of small turbot (less than 20 g) (Toranzo and Baja. 1990), other closely related vibrios (V. splendidus and V. pelagius) (Fouz et al., 1990) have recently become a risk in marine aquaculture (Lupiani et al., 1989: Toranzo et al., 1989).

Myxobacteria, pseudomonads and Gram-positive cocci have been sporadically found in gills, skin lesions and internal organs of turbot, but always in mixed infections with Vibrio species or parasites (Devesa et al., 1989). Toranzo et al. (1989) also reported the presence in Galicia of Aeromonas salmonicida and Renibacterium salmoninarum isolated from salmon and trout reared in sea water; these have not been detected in turbot.

Reports of viral infection in turbot are scarce, yet double-stranded RNA viruses have been isolated from turbot: infectious pancreatic necrosis virus (IPNV) serotype Ab in France (Gastric et al., 1987); IPNV serotype NI in Norway (Mortensen et al., 1990) and the turbot rotavirus (TRV) in Galicia (Lupiani et al., 1989). The only DNA virus, the herpes virus scophthalmi (Buchanam and Madeley, 1978), was found using electron microscopy.

There are few reports of parasitic infections in wild or cultured turbot. Ferguson and Roberts (1975) found 3-year-old cultured turbot in Scotland with lethal myeloid leucosis due to Haemogregarina sachai. Matthews and Matthews (1980) reported the microsporidian Tetramicra brevifilum which produced small xenomas that gave rise to large cystic formations in skeletal muscles of turbot along British coasts. Devesa et al. (1989) described the presence of the ciliate Cryptocaryon sp. associated with ulcers and tin lesions in cultured turbot from Galicia. Another parasite was the cestode Bothriocephalus scorpii which was reported to have a high prevalence in wild populations of turbot from the Galician coast (Sanmartin-Durán et al., 1989).

In this paper the preliminary results of a disease survey conducted in a turbot farm located in the Ria de Vigo (Spain), from December 1989 to September of 1990, are given. Several stocks of different origin were monitored, comparing apparently healthy

fish with those showing various pathological symptoms. Samples from imported specimens were also analyzed before the fish were released into the farm water tanks.

MATERIAL AND METHODS

A total of 225 fish (ranging from 6 g to 1.5 kg average body weight) were processed for bacteriological, virological, parasitological and histological studies.

For bacterial analysis, samples of kidney, liver and damaged skin were streaked onto plates of Bacto tryptic soy agar (TSA) (Difco) with 1% NaCl added, marine agar Z-2216 (Difco) and thiosulphate citrate bile salt (TCBS), (Difco) and they were incubated for 48 h at 22ºC. Pure cultures of the isolated colonies were subjected to taxonomic analysis using conventional morphological and biochemical plate and tube tests (Fouz et al., 1990) and the results were recorded after 4 days of incubation at 22°C.

For virus isolation, spleen and kidney were removed from the fish, pooled and processed using standard virological procedures (Amos, 1985). Chinook salmon embryo (CHSE-214) and rainbow trout gonad (RTG-2) cells maintained at 15ºC, and epitheliome papillosum cyprini (EPC) incubated at 25ºC, were used in the virological analysis. Cells were cultivated in 24-well tissue culture plates (Costar) with 10% newborn calf serum and containing 100 I.U. penicillin and 100 µg streptomycin per ml.

Smears of skin, muscle, gills and kidney were made and then examined using phase-contrast microscopy to detect parasitic infections.

For histology, liver, spleen and kidney were fixed whole in 1% glutaraldehyde and 4% formalin for 24 h and 2-3-mm-thick transverse sections were taken from each organ. Tissue sections were embedded in paraffin and 5-µm sections were stained with iron hematoxylin, acid fuchsin and aniline blue (Gray, 1954).

RESULTS AND DISCUSSION

The results of the bacteriological analysis are summarized in Table I. Bacterial diversity in imported fish (sampled just prior to their arrival at the farm) was lower than in the

turbot populations with and without clinical symptoms monitored along the survey. Although these two groups showed a similar spectrum of bacterial species, only V. anguillarum serotype 01 was isolated from diseased fish with a very low prevalence.

Although they can constitute part of the normal marine microflora, we have isolated members of the V. splendidus - V. pelagius group associated with mortalities of turbot, salmon and trout reared in sea water (Lupiani et al., 1989; Toranzo et al., 1989). In addition, we have recently demonstrated (Fouz and Toranzo, unpublished data) that some of these vibrios are V. damsela which was described as a common pathogen from fish, reptiles and mammals (Blake et al., 1980; Love et al., 1981). Occasionally, the external signs of these atypical vibrioses in turbot are lesions centered on the mouth and head which create clinical manifestations similar to the enteric redmouth syndrome of salmonids infected by Yersinia ruckeri.

Vibrio fisheri and V. harveyi were also detected in apparently healthy turbot and in diseased fish (Table 1). Until now, these species of Vibrio were isolated in Galicia mainly from complex pathological syndromes where a variety of different microorganisms (myxobacteria, parasites, viruses) or tumoral processes (Devesa et al., 1989, Lamas et al., 1990) were involved in the disease. We have not detected the halophilic pathogenic vibrios V. ordallii and V. salmonicida in our area.

Gram-positive cocci were abundant in all samples. The presence of Staphylococcus in fishes upon arrival at the farm could be due to manipulation during transport. Although epizootics caused by these bacteria have not been described in our country, infections by Streptococcus and Staphylococcus produced major losses in fish cultured in Japan (Kusuda and Sugiyama, 1981; Nakasugawa, 1983), the USA (Baya et al., 1990a) and South Africa (Bragg and Broere, 1986). Similarly the presence of Moraxella-Acinetobacter in turbot cannot be ruled out since strains of this group have been implicated in mortalities of Atlantic salmon (Salmo salar L.) in Norway (Roal and Hastein, 1980) and striped bass (Morone saxatilis) in the USA (Baya et al., 1990b).

We have isolated Birnaviruses from only two samples. IPN-like viruses were isolated in routine surveys from turbot cultured in different farms in Galicia without causing mortalities (Novoa et al., 1991). In addition, five samples displayed a slow cytopathic

effect (cell lysis) in the RTG-2 cell line which persisted after several passages. However, the virus(es) have not yet been identified. Development of new marine cell lines could improve the isolation of other viral agents from turbot which may be unable to replicate in conventional cell lines.

Only 0.8% of the animals observed had Costia. No mortalities were detected in the tanks where this parasite occurred. However, costiasis can be an important disease in sea water since heavily infested salmon from sea cages suffered acute hyperplasia and fusion of the secondary lamellae, resulting in 40% mortality (Foppe and Hastein, 1982).

Only 2 of 225 fish examined had a microsporidian infection. Characteristic white cysts occurred in the liver and no spores were observed in any other tissues. Microsporidia are a potential threat for turbot culture because heavy infections in some species can lead to the inactivation of a substantial proportion of the body musculature and impairment of swimming (Matthews and Matthews, 1980).

Species of the genus Trichodina (Ehrenberg, 1931) are the most frequently encountered ciliates on the surface of marine fish. and trichodiniasis has been a point of concern in flatfish culture (Pearse, 1972; McVicar, 1978). Although Trichodina was usually found in skin smears of cultured turbot, this parasite did not cause any apparent pathology. The other ciliate, Cryptocaryon sp., was associated with skin ulcers and tin lesions. Devesa et al. (1989) reported that this parasite occurred only in fish with skin lesions. The prevalence of these lesions was 4% in the juveniles (less than 5 cm total length).

The cestode Bothriocephalus scorpii was found only in adults of commercial size, and with a low prevalence. The origin of the infection could be the fresh fish that is fed to cultured turbot since it has been reported (Sanmartin-Duran et al., 1989) that prevalence of this parasite in wild populations of turbot is very high.

This study is a preliminary overview of what is present in an ongrowing turbot aquaculture facility, in order to obtain base-line data useful for farmers. It must be noted that the findings reported here belong to a particular turbot farm and therefore they are a consequence of water and fish food quality or management conditions. They cannot be

extrapolated to other turbot farms. The potential pathogenic capacities of the microorganisms isolated in the present study are currently being investigated.

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TABLE 1

Bacterial species	Fish sampled after transport	Apparently healthy fish	Diseased fish
V. anguillarum	-	-	+
V. splendidus	-	++	+++
V. pelagius	++	++	+++
V. fischeri-harveyi	-	++	+
Vibrio sp.	-	+++	++
Pseudomonas	++	++	+
Acetinobacter/Moraxella	+	+	+
Streptococcus	-	++	++
Staphylococcus	+++	-	-

Incidence: + + +, in 76-100% of fish sampled; + +, in 20-75% of the fish; +, in <2O% of the fish.